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## Intestinal Microbiota And Diet in IBS: Causes, Consequences, or Epiphenomena?

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**Irritable bowel syndrome (IBS) is a heterogeneous functional disorder with a multifactorial etiology that involves the interplay of both host and environmental factors. Among environmental factors relevant for IBS etiology, the diet stands out given that the majority of IBS patients report their symptoms to be triggered by meals or specific foods. The diet provides substrates for microbial fermentation, and, as the composition of the intestinal microbiota is disturbed in IBS patients, the link between diet, microbiota composition, and microbial fermentation products might have an essential role in IBS etiology. In this review, we summarize current evidence regarding the impact of diet and the intestinal microbiota on IBS symptoms, as well as the reported interactions between diet and the microbiota composition. On the basis of the existing data, we suggest pathways (mechanisms) by which diet components, via the microbial fermentation, could trigger IBS symptoms. Finally, this review provides recommendations for future studies that would enable elucidation of the role of diet and microbiota and how these factors may be (inter)related in the pathophysiology of IBS.**

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## INTRODUCTION

With a prevalence of approximately 10–15%, irritable bowel syndrome (IBS) is one of the most common gastrointestinal (GI) disorders in the industrialized world (1). The high prevalence together with the reduced quality of life and associated co-morbidities in patients suffering from IBS put a significant negative burden on both patients and society (2). IBS is a heterogeneous functional disorder and commonly subtyped according to the prevailing bowel habit into IBS with constipation (IBS-C), IBS with diarrhea (IBS-D), mixed IBS with both constipation and diarrhea and unsubtyped IBS with neither constipation nor diarrhea using the Rome III criteria (3).

Although the etiology of IBS is incompletely understood, it is generally regarded as a multifactorial disorder involving the interplay of both host and environmental factors, including diet. The host factors include central factors, such as aberrant stress

responses, psychiatric co-morbidity, and cognitive dysfunctions, whereas intestinal functions are also involved, e.g., dysmotility, visceral hypersensitivity, low-grade immune activation, altered barrier function, and the intestinal microbiota composition (4).

During recent years, perturbations in the intestinal microbiota are increasingly being linked to the pathophysiology of IBS. The increased risk of new onset IBS after an episode of gastroenteritis (5) and the association with prior antibiotic use (6) support the importance of the intestinal microbiota in IBS. Numerous studies have demonstrated altered microbial profiles in (specific subgroups of) IBS patients compared with healthy individuals (7–11). The intestinal microbiota strongly interacts with exogenous factors, in particular diet, which may also directly or indirectly provoke IBS symptoms.

In this review, we describe the current evidence regarding the impact of diet and the intestinal microbiota and their (inter)relation on the pathophysiology of IBS.

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## THE IMPACT OF DIET ON THE INTESTINAL MICROBIOTA

The fetal intestine is thought to be sterile, although some recent studies indicate that colonization may already start before delivery, by bacterial transmission through the placental barrier (12,13). The colonization during and upon delivery involves a succession of microbial populations, a seemingly chaotic process strongly influenced by birth mode and infant feeding. Vaginally born infants are initially colonized by maternal fecal and vaginal microbes, such as *Bifidobacterium*, *Bacteroides*, *Prevotella*, and *Lactobacillus* spp., whereas infants born by cesarean section are inoculated by typical skin bacteria and bacteria from the hospital's environment (14,15).

The strong impact of diet on the indigenous microbial communities can already be observed even before weaning. Compared with formula-fed infants, the microbiota of breastfed infants is less diverse and more dominated by *Bifidobacterium* spp. (16–18). This is generally attributed to unique bioactive compounds such as human milk oligosaccharides that serve as metabolic substrates for a limited number of microbes (19), although breast milk itself also harbors a transmissible microbiota (20). A rapid diversification of the infants' intestinal microbiota and shift toward an adult-like microbiota occurs with the introduction of solid foods (21,22); yet, the maturation of the microbiota appears to continue in parallel to physiological development of, for example, the gastrointestinal tract and the central nervous system, throughout childhood and adolescence (23,24).

Once established, the intestinal microbiota appears to be relatively stable within individuals over time (25), whereas high variability between individuals is observed (26–29).

Pronounced differences in the composition and functional capacity of the human microbiota between geographically distant populations point toward an important role of the diet in these inter-individual variations (26,30–32). The microbiota in non-Western populations, consuming a diet high in plant-derived carbohydrates, has consistently been shown to be more diverse and enriched in *Prevotella* spp. at the expense of *Bacteroides* spp. as compared with Western populations consuming a diet high in animal protein, sugar, starch, and fat (reviewed in (33)). It remains to be determined to what extent the microbiota composition is shaped by the diet, as the impact of genetics, ethnic, and cultural factors, such as hygiene and living conditions, cannot be ruled out in such studies comparing distinct populations.

Nevertheless, habitual long-term dietary patterns have also been directly linked to intestinal microbial enterotypes. Especially, protein and animal fat intake has been associated with the *Bacteroides* enterotype, whereas a high carbohydrate intake was associated with the *Prevotella* enterotype (34). The effect of short-term dietary interventions on the microbiota composition appears to be only modest (34,35), unless the intervention comprises an extreme switch in diet (36–38). David *et al.* (37) demonstrated that even within days after the transition to a diet entirely composed of animal or plant products, changes in microbial structure, metabolic activity, and gene expression can be observed. In subjects receiving the animal-based high fat and protein diet, the abundance of

bile-tolerant bacteria (*Alistipes*, *Bilophila* and *Bacteroides*) together with branched short-chain fatty acids indicative of amino-acid fermentation increased, whereas levels of bacteria metabolizing dietary plant polysaccharides (*Eubacterium rectale*, *Roseburia*, *Ruminococcus bromii*) decreased. In agreement, switching to a diet high in resistant starch has been shown to increase *Ruminococcus bromii* and *Eubacterium rectale* related species (39), which are known for their saccharolytic properties. Altogether, these studies indicate that the intestinal microbial community structure as well as its function and metabolic output are influenced by our diet, especially in the case of clearly distinct dietary patterns.

## INFLUENCE OF DIET ON IBS

Surveys on perceived food intolerance show that 64–89% of IBS patients report their symptoms to be triggered by meals or specific foods (40–42). The majority indicate that they limit or exclude certain foods without professional counseling (40,42), thereby increasing the risk of inadequate dietary intake. Monsbakken *et al.* (40) found indications for inadequate dietary intake in 12% of IBS patients studied, whereas others found adequate or even increased intake of some nutrients (43–45). Overall, data on habitual dietary intake are limited and may depend on the study population included. Meal-related symptom aggravation is further supported by studies showing a postprandial worsening of pain using symptom diaries for 6 weeks (46) or rectal barostat (47,48).

Foods often reported to provoke symptoms include wheat/grains, vegetables, milk products, fatty foods, spicy foods, coffee, and alcohol (40–42,49) and are especially reported to be associated with abdominal pain and gas problems (41,42). Overall, the percentage of responders on exclusion diets varies from 15 to 71% (50), but it has to be noted that most studies suffer from major methodological limitations and only a minority of subjects was found to react positively to double-blind food challenges (51–53).

Although placebo and possibly nocebo effects have to be considered in IBS, possible physiological mechanisms of (perceived) food intolerance include an exaggerated sensory and motor response and/or incomplete absorption, which may lead to symptoms in a susceptible host. High fat intake, for example, is associated with an exaggerated colonic motor response to eating and increased visceral sensitivity in IBS patients, whereas duodenal lipid infusions delay small intestinal transit. Randomized controlled trials adjusting fat intake are, however, limited (54). Poorly absorbed carbohydrates, like lactose, fructose, and galacto- or fructo-oligosaccharides (fructans), can result in luminal distension by osmotic effects and increased gas production due to microbial fermentation. Although this is a normal physiological phenomenon, it can result in symptoms in subjects with an altered microbiota, increased visceral sensitivity, and/or abnormal gas handling. A recent study by Yang *et al.* (55) found increased mucosal mast cell numbers, serum tumor necrosis factor- $\alpha$ , rectal sensitivity, and anxiety in lactose intolerant IBS-D patients, supporting that neuro-immune modulation of visceral function is a potential underlying mechanism. Perceived intolerance to lactose, fructose, and fructans is frequently

reported and uncontrolled studies point to symptom improvement after removal of milk containing products or wheat from the diet (42). However, these studies should be interpreted with caution because of a large placebo and nocebo response in IBS. The overall evidence for an increased incidence of lactose intolerance in IBS patients was found to be weak and to be moderate—weak for the benefit of low lactose intake in these subjects (56).

Evolving from previous studies on lactose and fructose intolerance, a more generalized approach of intolerance to poorly absorbed and rapidly fermented carbohydrates has been introduced: FODMAPS, including fermentable oligo-, di- and monosaccharides and polyols. Both retrospective and prospective open studies have shown reduced symptoms after introduction of a low-FODMAP diet in IBS patients with suspected or proven fructose or lactose malabsorption (57,58). These findings are confirmed by randomized controlled trials, showing a significant improvement of overall symptoms, abdominal pain and bloating, in patients with bloating and/or diarrhea (59) and in small ( $n = 15-30$ ) unselected IBS patient groups (60,61). One study showed recurrence of symptoms on re-challenge with fructose and/or fructans (62). Involvement of gas production is supported by findings that IBS patients produced more hydrogen relative to healthy controls and lacked an increased methane production on a high vs. low-FODMAP diet (60). However, it should be noted that the long-term benefit of a low-FODMAP approach in large (unselected) patient groups is unclear, especially considering its invasiveness, which requires strict guidance by a dietician, and the potential risks of, for example, reduced fiber intake and changes in microbiota composition and activity (59,63). Furthermore, randomized controlled trials are needed to prove whether it is superior to other dietary interventions (e.g., lactose or fructose reduction) or general dietary advice (e.g., the NICE guidelines). Finally, the FODMAP levels vary between studies, countries, and products, which should be taken into account when interpreting study results and considering low-FODMAP diets.

Foods can also evoke symptom onset by immune activation or altered neuro-endocrine responses (42). Food allergy or intolerance associated with IgE-mediated immune responses is uncommon and evidence for IgG/IgG4-mediated hypersensitivity is inconclusive (42,64). However, in subsets of IBS patients, increased numbers of T-lymphocytes, mast cells, eosinophils, and/or enteroendocrine cells have been found (65–67), but their exact role in food intolerance and symptom generation is unclear.

Interest in the role of gluten intolerance (and the possible benefit of gluten-free diets) is increasing. A systematic meta-analysis found a pooled prevalence for celiac disease up to 4% among IBS patients (68). The prevalence of non-celiac gluten sensitivity among unselected IBS patients is unknown and complicated by clear diagnostic criteria and overlap in symptoms. Although symptom reduction after a gluten-free diet (69) and worsening of symptoms after a gluten challenge (70) have been reported, the recent study Biesiekierski *et al.* (71) found no symptom induction by giving pure gluten to IBS patients following a gluten- and FODMAP-free diet. These findings suggest that not gluten but fructans and/or other components might contribute to symptoms in perceived

“wheat” intolerance. Furthermore, Carroccio *et al.* (72) showed that the majority of IBS patients diagnosed with wheat sensitivity had multiple food sensitivities.

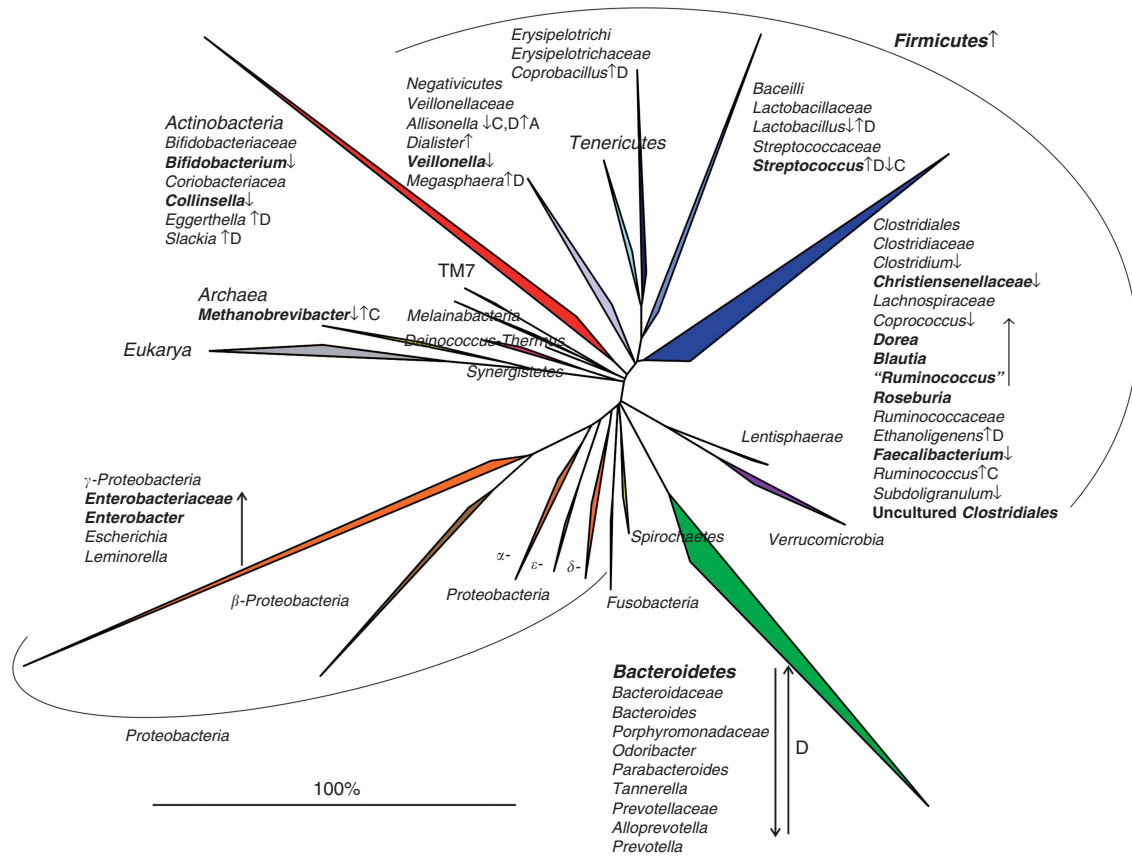
Dietary supplementation studies focus on probiotics, prebiotics, synbiotics, and fiber intake. A recent meta-analysis on the use of probiotics shows significant effects on subjective global symptom improvement, but study heterogeneity is statistically significant and no conclusions can be drawn on which individual species or strains are most beneficial (73). Studies on prebiotics and synbiotics are limited and insufficient to draw conclusions. Evidence for the benefit of dietary fiber was found for soluble fiber intake only (74), but it should be noted that overall study quality was moderate and more studies are needed on its effects in IBS subgroups.

Although several food components are associated with symptom generation, detailed analyses on the effect of food intake on symptom generation and underlying mechanisms are limited. In addition, many studies suffer from methodological limitations, in part inherent to the complexity of food research.

## MICROBIOTA ALTERATIONS IN IBS

Following the first comprehensive analysis reporting a distinctive intestinal microbiota composition in IBS patients (75), several studies have identified differences between the microbiota of IBS patients, or subgroups thereof, and healthy controls (reviewed in (7)). Recently, clinical guidance regarding the modulation of intestinal microbiota in IBS was provided by the Rome Team Working Group (76), which concluded that there is good evidence supporting the concept that the intestinal microbiota is perturbed in patients with IBS. However, despite a growing consensus regarding an association between the intestinal microbiota and IBS, results of current studies lack general consensus and a specific microbial signature in IBS remains elusive (**Figure 1**). Lack of detailed phenotypic characterization of patients, small sample sizes, and the cross-sectional study designs (providing only a single snapshot of the microbiota composition) in the majority of studies may all contribute to the fact that the markers of the IBS microbiota reported in several studies (marked in bold on **Figure 1**) are not reproducibly detected in all cohorts. Moreover, exogenous factors, including diet, are most often not taken into account, despite the demonstrated effect of diet on the microbiota and the potential alterations in diet associated with IBS. Finally, it is noteworthy that Jeffrey and colleagues identified distinct subsets of IBS patients, not corresponding to the traditional IBS subtypes, with an altered or normal-like microbiota composition (8), indicating that the disturbed microbiota might be relevant for the pathology of part of the IBS patients. Moreover, those with a normal-like microbiota had more adverse psychological factors, suggesting that central factors may predominate over microbiological factors in some but not all IBS patients.

Several recent comprehensive studies of the microbiota in IBS have reported an increase in the relative abundance of *Firmicutes*, mainly *Clostridium* cluster XIVa and *Ruminococcaceae*, together with a reduction in the relative abundance of *Bacteroidetes* (7–9,77). Bifidobacteria have been shown to be depleted in both



**Figure 1.** Phylogenetic tree of the human intestinal microbiota with indicated microbial groups that are significantly altered in irritable bowel syndrome (IBS) patients relative to controls. The increase and decrease in microbial groups in IBS patients are marked by arrows facing up and down, respectively, and in bold if reproduced by at least two independent studies. If microbial groups are altered only in diarrhea or constipation predominant IBS patients, letter D or C in superscript follows the arrows, respectively. The figure is generated on the basis of the data published in (8–11,75,77–79,82,85,98,116).

fecal and mucosal samples of IBS patients (9,78–81), which adds to the trend toward a modest beneficial effect of *Bifidobacterium* supplementation, in terms of the improvement of global IBS symptoms and pain scores as reported in a meta-analysis of probiotic trials.(73) A lower diversity and a higher instability of the (*Firmicutes* fraction or function of the) microbiota in IBS patients relative to controls have been reported (82–85), although these findings need confirmation in studies with sufficient depth of the microbiota analysis.

Most if not all studies that compare microbial profiles in IBS patients and healthy controls are associative and cannot distinguish cause from consequence. However, evidence of a potential causal role for the GI microbiota is supported by a fecal transplantation experiment in which visceral hypersensitivity, presumed to underpin abdominal pain in a subgroup of IBS patients, could be transferred via the microbiota of IBS patients to previously germ-free rats (86). Furthermore, germ-free mice colonized with the microbiota from IBS-D patients have been shown to exhibit a faster GI transit and impaired intestinal permeability as compared with mice gavaged with the microbiota from healthy humans (87). The mechanisms through which the microbiota exerts these effects are not fully understood, but the fact that supernatants from colon biopsies

of IBS patients, with increased levels of histamine and proteases, excite human submucosal neurons could indicate a brain–gut axis connection (88). Furthermore, an increase in fecal serine proteases has been linked with symptom development in IBS patients (89,90). Although the origin of both histamine and proteases could be both human and microbial, it is noteworthy that some of the *Firmicutes* bacteria that were found to be increased in abundance in IBS patients (8,9) are known to secrete large amounts of extracellular proteases (91). Alternatively, the predominantly endogenous fecal proteases might not be degraded by microbes because of the accelerated transit (as seen in IBS-D patients) or because of the disturbed metabolic activity of the microbiota (92). Serine protease inhibitors, the antagonists of serine proteases, are produced by many bacteria, including bifidobacteria (93), which may contribute to the possible benefit of bifidobacterial supplementation.

Dysregulated intestinal immune function, chronic low-grade mucosal inflammation, and increased mucosal permeability and barrier dysfunction have all been suggested as putative pathogenic mechanisms in IBS, in which the intestinal microbiota might have a role (reviewed in (76,94)). Moreover, the bidirectional interactions between the intestines and the central nervous system, which have an important role in the pathogenesis of IBS,



have suggested to be modulated by the microbiota (reviewed in (95)). Yet, there is a lack of data on the exact mechanisms through which the host-microbiota interactions underlie pathophysiology and generate symptoms. Identifying these mechanisms is further complicated by the fact that the majority of GI microbes remain uncharacterized (96). Indeed, most putative microbial markers of IBS are among uncultured bacteria. For example, uncultured bacteria related to *Ruminococcus torques* are significantly enriched in IBS patients in several cohorts (9,11,75), and their abundance positively correlates with bowel symptoms (9,10,97). It is intriguing that this bacterial group has been shown to be suppressed following a multispecies probiotic treatment that alleviated IBS symptoms (98). Similarly, uncultured bacteria within the *Clostridiales* order are reproducibly detected in significantly depleted abundance in IBS (9,10) but also in ulcerative colitis (99). Focus on the function of these uncultured bacteria should be of major interest for future studies.

### MICROBIOTA-DIET INTERACTIONS IN IBS

Intestinal microbes have an important role in the digestion of dietary components, resulting in metabolites that may directly or indirectly contribute to IBS symptoms (Figure 2).

Colonic fermentation of carbohydrates mainly results in short-chain fatty acids (SCFAs, including acetate, propionate, and butyrate) and variable amounts of hydrogen and carbon dioxide. Several low-abundant microbial groups can subsequently dispose the produced hydrogen into acetate, hydrogen sulfide, and methane. The fermentation of protein residues, primarily in the distal colon, produces a variety of metabolites, including ammonia, organic acids, heterocyclic amides, and phenolic and indolic compounds, that are toxic and detrimental to gut health (100). Moreover, fat and digested proteins increase the excretion of bile acids that are subject to extensive bacterial transformation in the intestinal tract. This could be of particular relevance as bile acids inhibit many bacteria and may modulate microbiota composition and also have the potential to affect major pathophysiological factors in IBS, including GI motility, secretion, and immune function (101,102). Indeed, in a recent large-scale study, increased colonic bile acid exposure was demonstrated in a subset of, predominantly non-constipated, IBS patients correlating with diarrhea and accelerated colonic transit (101).

An association between the intestinal microbiota and protein metabolism has been established by a significant correlation between the abundance of several post-infectious (PI)-IBS microbial markers and host amino-acid metabolism (10). Among harmful products of protein fermentation (100,103), hydrogen sulfide might be relevant for compromising intestinal health as it directly impairs epithelial metabolism (104) and acts as a gut-brain signaling molecule (105). Hydrogen sulfide can be converted to thio-sulfate and further oxidized to tetrathionate during inflammation. The latter supports the growth of *Salmonella* (106) and other tetrathionate utilizing pathogens from the Gamma-Proteobacteria class (107), many of which have been associated with bowel symptoms of IBS patients (9,10,82).

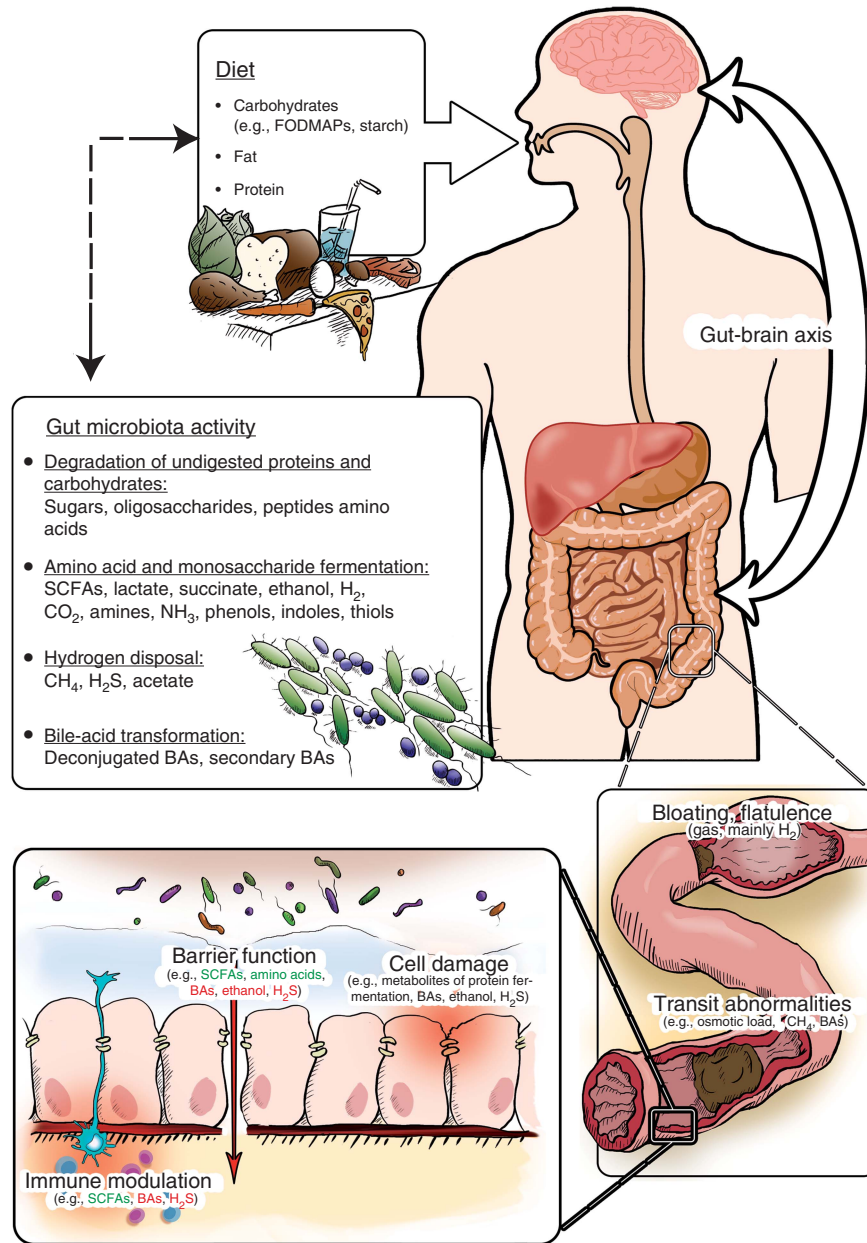
FODMAPs, indigestible carbohydrates, can be fermented by intestinal microbes, resulting in increased gas production and an osmotic effect that can provoke IBS symptoms in a sensitive host (108), although similar changes may go unperceived in normal subjects (109). Carbohydrate utilizing GI bacteria such as *Dorea* spp., major gas producing bacteria in the human intestine (110), were reported to be significantly increased in abundance in IBS patients (9,11). Overproduction of gas, specifically hydrogen, has been associated with IBS symptoms (111), especially abdominal pain and flatulence. In patients with IBS-D, whose colon was found to be less able to accommodate the increased intestinal volume (112), overproduction of gas could be a trigger for increased colonic wall tension. In general, hydrogen produced in the gut is most efficiently removed by the methanogenic archaea (113) that appear to be depleted in IBS-D patients (9,10) and increased in IBS with constipation patients (reviewed in (114)). Although there is substantial evidence for implication of methane in IBS symptomatology, most data are based upon methane breath testing. Hence, future studies, especially those addressing the diet-microbiota connection in IBS, should quantify methanogens to provide more direct evidence.

Alternative pathway for gas elimination is the conversion into acetate by acetogenic bacteria, *Blautia* spp. (115). *Blautia* spp. are highly prevalent and dominant intestinal bacteria, which have been found to be elevated in IBS patients (9,79). Intriguingly, the major fermentation end-product—acetate—has been reported as significantly increased in IBS patients (116), although this was not confirmed in another study (117). This may relate to the fact that most studies measure stool concentrations without taking the total stool volume into account, thereby potentially concealing overall increased acetate production as a result of dilution due to increased stool volume.

SCFAs produced upon the microbial fermentation of undigested carbohydrates and reduced levels thereof may also contribute to symptom generation in IBS. SCFAs have some documented health benefits, in particular butyrate, which is an important energy source for colonocytes, and among others inhibits inflammation and enhances barrier function (118). Moreover, intraluminal administration of butyrate into the distal colon has been shown to decrease visceral sensitivity in healthy humans (119). However, excessive intake of fermentable carbohydrates and high SCFA levels can also increase the osmotic load and thereby lead to diarrhea (120).

Data on SCFAs in IBS are limited and inconsistent, showing no alterations, increased or decreased levels of SCFAs relative to controls (61,116,117,121). Furthermore, altered SCFAs profiles might merely reflect diet rather than a feature of the condition. Given that the major products of FODMAPs fermentation are SCFAs, and that some major microbial SCFA-producers may be altered in IBS, the connection between FODMAPs, SCFAs, microbiota, and IBS symptoms calls for further elucidation.

Few studies have examined the impact of dietary interventions, in particular FODMAP restriction, on the microbiota in IBS patients. A low-FODMAP diet has been linked to reduced bifidobacterial counts (59), which seems a paradox given their potential health benefit. A recent study reported a reduction in total bac-



**Figure 2.** Non-exclusive listing of dietary metabolites that can be regulated by microbial activity and contribute to irritable bowel syndrome (IBS) symptoms. The impact of metabolites affecting different domains of intestinal health is depicted; however, it should be noted that the effects of some metabolites depend on their concentration or further conversion. Note: for immune modulation and barrier function, metabolites with positive and negative effects are colored in green and red, respectively. SCFAs, short-chain fatty acids; BAs, bile acids.

terial abundance following the introduction of a low-FODMAP diet, as well as a reduction in absolute counts but not relative abundance of specific bacterial groups, including bifidobacteria as compared with habitual diet (63). However, as compared with a diet high in FODMAP content, the low-FODMAP diet appeared to be associated with marked lower relative abundances of butyrate-producing bacteria and *A. muciniphila*, and a significantly higher abundance of *R. torques*, adding more paradoxical data on the effects of low-FODMAP diet (63).

Extensive analyses of microbiota composition, functionality, and fermentation products in relation to FODMAP restriction and symptom generation are currently, however, lacking.

The direct impact of diet-microbiota interactions on IBS symptoms has been demonstrated in an elegant study on the impact of a diet rich in fermentable substrates. The introduction of this flatulogenic diet significantly increased the gas volume and the number of gas evacuations, as well as abdominal symptoms and digestive discomfort in both healthy subjects and flatulent

patients (122). Yet, upon dietary challenge, patients' microbiota developed instability in composition, exhibiting variations in the *Bacteroidetes* and *Firmicutes* phyla and reduction in microbial diversity, whereas healthy subjects' microbiota remained stable. Hence, the extent of dysbiosis in IBS patients likely depends on their diet. In a small study among children with IBS, the introduction of a low fermentable substrate diet significantly decreased abdominal pain severity and frequency in some but not all children. Responders to this dietary intervention appeared to differ in intestinal microbiota composition at baseline from nonresponders, indicating that the efficacy of dietary interventions might be influenced by the patient's microbiota (123). To prove the validity, these pilot results will have to be replicated in larger studies.

## CONCLUSION, RECOMMENDATIONS FOR FUTURE RESEARCH

Both experimental and observational studies provide good evidence to conclude that microbial alterations frequently observed in IBS patients potentially drive or perpetuate gastrointestinal symptoms. Yet, the strong interaction between distinct dietary patterns and the intestinal microbial communities is likely one of the explanations for the inconclusive findings among studies comparing the microbiota composition in IBS patients and healthy subjects.

More well-designed studies on the effect of food intake on symptom generation and the underlying mechanisms are warranted. The potential benefit of FODMAP restriction seems promising but requires further study and dietary guidance is essential given the complexity and risk of inadequate nutrient intake. In addition, more evidence on long-term outcome and well-performed challenge or re-introduction trials are warranted as important adaptations may occur. The incorporation of microbiota characterization in such studies would both provide insight into the impact of these dietary interventions on the microbiota, as well as offer the opportunity to examine whether the microbiota can predict response.

Given that IBS patients comprise a heterogeneous group both in the type of bowel symptoms and food components to which they might respond, and that the microbiota is extremely complex and variable between individuals, it is clear that defining the correlations between IBS, microbiota, and diet is not an easy task. However, high-throughput molecular methods enable reproducible and in-depth analysis of the intestinal microbiota, providing the necessary tool for testing these correlations. The major challenge remains to include the conscientious collection of dietary information in future studies in this field. Moreover, future studies should include multiple microbiota analysis at various time points in a large number of well-phenotyped patients to increase our insight in the contribution of intestinal microbiota perturbations to the diverse and fluctuating symptom pattern in IBS patients.

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## CONFLICT OF INTEREST

**Guarantor of the article:** John Penders, PhD.

**Specific author contributions:** The initial drafting of the manuscript was prepared by Mirjana Rajilić-Stojanović, Daisy M. Jonkers, Robin C. Spiller, Anne Salonen, Kurt Hanevik, Jonna Jalanka, Chaysavanh Manichanh, Elena Philippou, Gerard Clarke, and John Penders.

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